The following information was submitted on 12/29/2006:

Name & Sponsoring Organization

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Are you submitting comments on behalf of a sponsoring organization? No
If yes, please enter the name of the organization:

Comments and Questions

1. Do you have comments on the priority areas for the development and validation of alternative test methods listed above?

   Not Provided.

2. Considering available science and technology, what development, translation, and validation activities are most likely to have the greatest impacts within the next five years on refining, reducing, or replacing animal use?

   Comments addressed to priority area number 8 (Neurotoxicity) The development of alternative methods for the assessment of the capability of organophosphorus compounds (OPs) to induce organophosphorus induced delayed polyneuropathy (OPIDP) would be strongly desirable. There are overwhelming scientific evidences that the OPIDP is triggered by the modification of a membrane protein called neuropathy target esterase (NTE). The biological function of this protein remains unclear, although in the last few years data have been reported suggesting a critical role in the embryonic development. NTE has been detected in brain of all vertebrates and in several cellular lines (chromaffin cells, lymphocytes, neuroblastoma, glioma, &). However, the assays for determining NTE, as well assays of NTE inhibition and reactivation, are well standardised only in hen brain (animal model employed in the officials guidelines for testing OPIDP). The official protocols of UE (B.37 and B.38), OCDE (418 and 419 Guidelines) and EPA (870.6100 Harmonized Test Guideline) for the assessment of the delayed neurotoxicity of OPs incorporate two independent toxicity assays. The assay for acute exposure requires a minimum of 28 days of observation and 48 animals. The assay for repeated dose is performed after the acute test and requires a minimum of another 48 days of observation and 48 animals. NTE expressed by chromaffin cells is a very promising candidate for being used as in vitro model for studying the enzyme. Indeed, the total NTE activity is the highest among all tested cellular systems, the activity is stable along the whole life of the culture and specially, the response to OPs inhibition is similar to response reported by NTE of hen brain in vivo assays. Other systems with possible theoretical advantages over chromaffin cells (not primary cultures, human origin, etc.) are also promising, although the degree of characterization of the enzyme is not so high as chromaffin cells. The objective of preparing and arranging a protocol for testing the capability of OPs to induce OPIDP using as endpoint the inhibition and reactivation of NTE expressed in chromaffin cells in culture is easily reachable in the short term (1-1.5 years). The possibility of use other cellular systems also theoretically suitable for this purpose, as human neuroblastoma, human lymphocytes or even recombinant human brain NTE should be scheduled by medium term, since the characterization of NTE in these systems is not so advanced as in chromaffin cells. Potential benefits of the development of

the proposed alternative methodology Current tendencies in the research and treatment of Alzheimer disease are focused towards the design of specific inhibitors of cholinesterase, as organophosphorus molecules. Therefore, in vitro methodologies for the fast and reliable detection of neuropathic organophosphorus compounds during the first stages of the development are needed. This application would be also suitable in the case of development of new OPs pharmaceuticals to be used as antihelminthic. The developed methodology would be also extrapolated to the evaluation of neurotoxicity of other molecules with OPs structure (essentially insecticides).

3. What research and development activities hold the greatest promise in the long-term for refining, reducing, or replacing animal use?

   Comments addressed to priority area number 6 (Reproductive/developmental toxicity) Neuropathy target esterase is an esterase found in nervous system which seems to own a dual physiological role. In adult individual seems to be in close relationship with homeostasis of membrane phospholipids and its inhibition by organophosphorus compounds and further modification is the tiger of the so called organophosphorus induced delayed polyneuropathy. By the other way, NTE seems to be critical during organogenesis since embryo of mice lacking of NTE are not viable and embryo deficient in one NTE allele are viable, although the animal borns with serious neurological deficiencies (hyperexcitability and others). NTE expressed by chromaffin cells is a very promising candidate for being used as in vitro model for studying the enzyme. Indeed, the total NTE activity is the highest among all tested cellular systems, the activity is stable along the whole life of the culture and the response to OPs inhibition is similar to response reported by NTE in vivo assays. To our opinion, cellular models expressing NTE could be a great promise in the long term for being used in the testing of developmental toxicity.

4. What are appropriate measures for evaluating progress in enhancing the development and use of alternative test methods?

   Not Provided.